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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#11

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TITLE	:	ALLERGEN/INFLAMMATORY TESTING AND DIAGNOSIS
EXAMINER	:	RON SCHWADRON, Ph.D
ART UNIT	:	1644

SUPPLEMENTAL REPLY TO THE FIRST OFFICE ACTION
AND REQUEST FOR PERSONAL INTERVIEW

Honorable Commissioner for Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

In further reply to the First Office Action filed August 17, 2000, enclosed herewith is a clear copy of the enclosures submitted with the initial reply.

Furthermore, as previously indicated, both the inventor Brigit Helm and the commercial representative for the Applicant, Dr. Penny Wilson would like to arrange for a personal interview with the Examiner. They are tentatively planning a trip to the

U.S. between September 25, 2000 and October 25, 2000. It would be appreciated if the Examiner could telephone the Applicant's undersigned attorney and advise if he is available within this time frame for an interview and whether there are any specific dates which are not convenient.

Respectfully submitted,

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F:\G&B\Z\815\25A\AMEND.SUP

I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on September 11, 2000

By: _____ Dated: September 11, 2000
Thomas M. Galgano, Esq.

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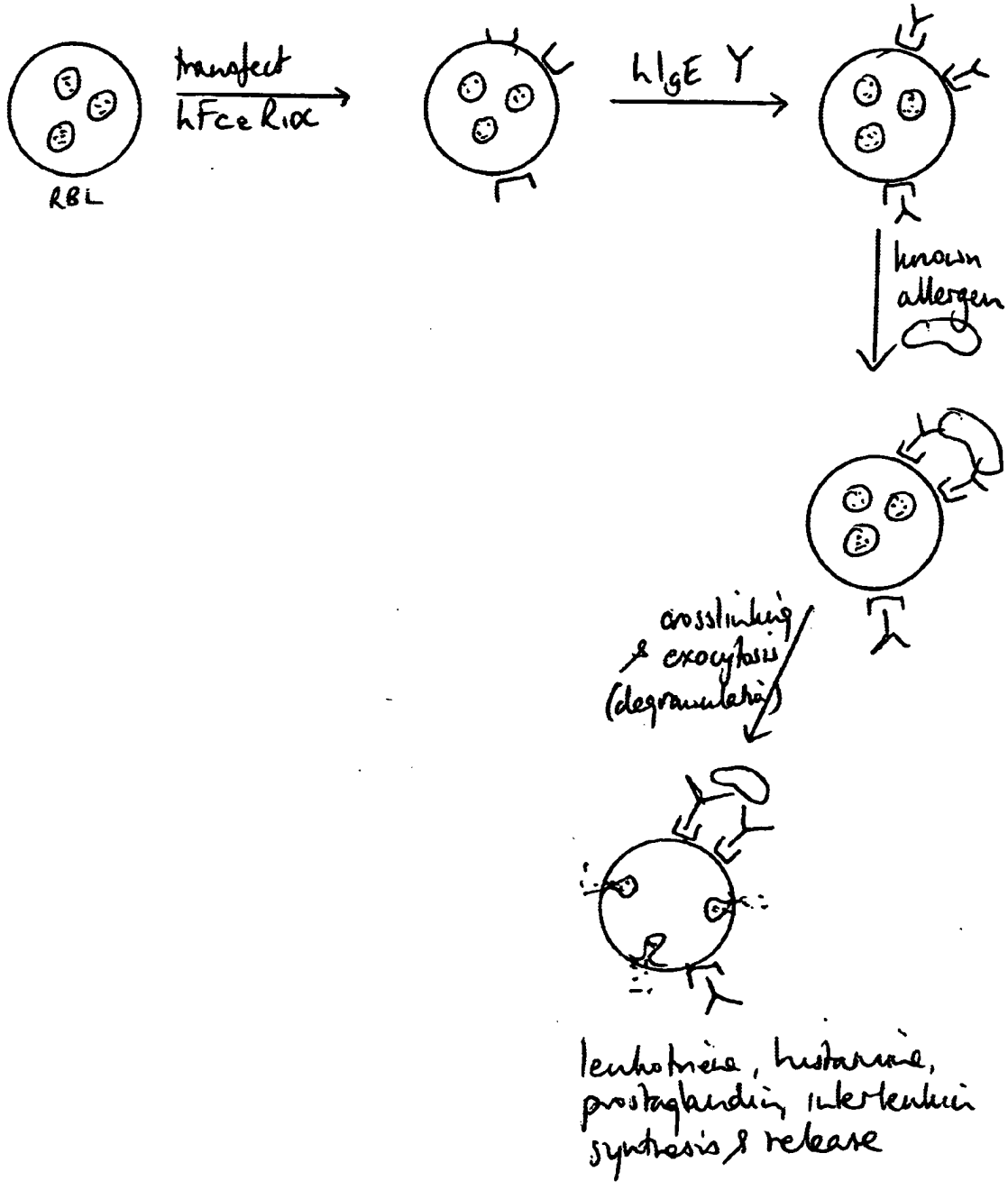

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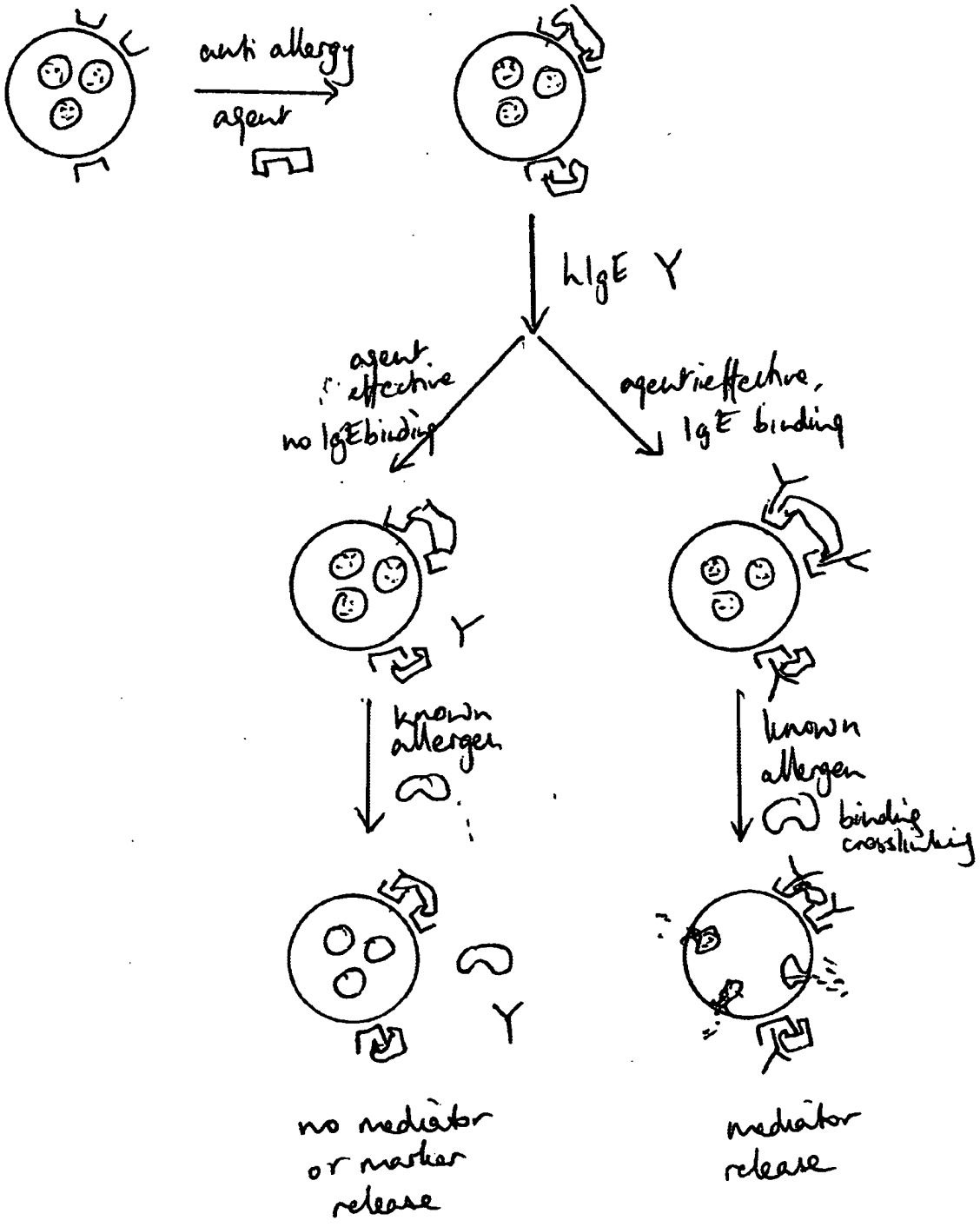

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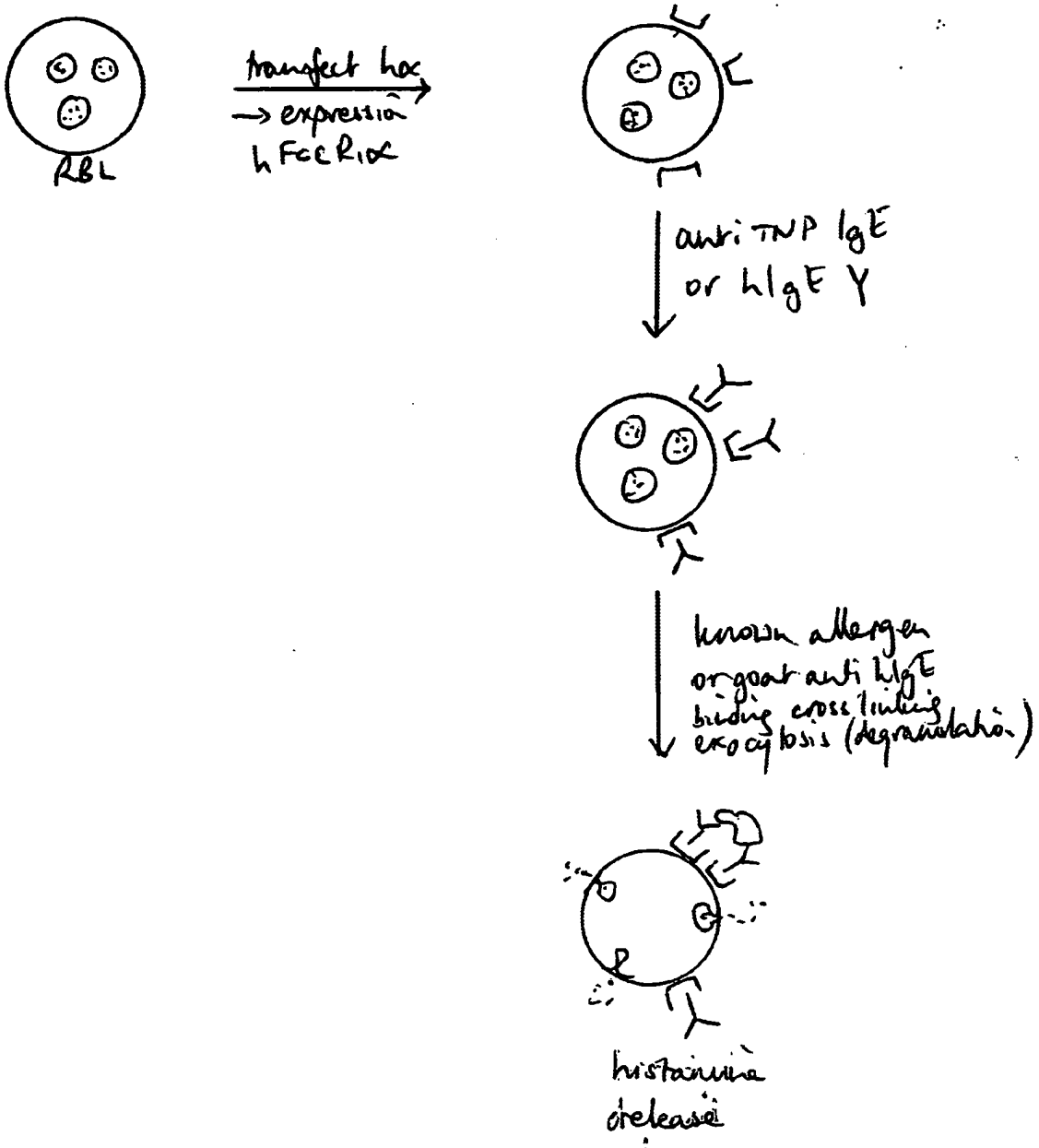

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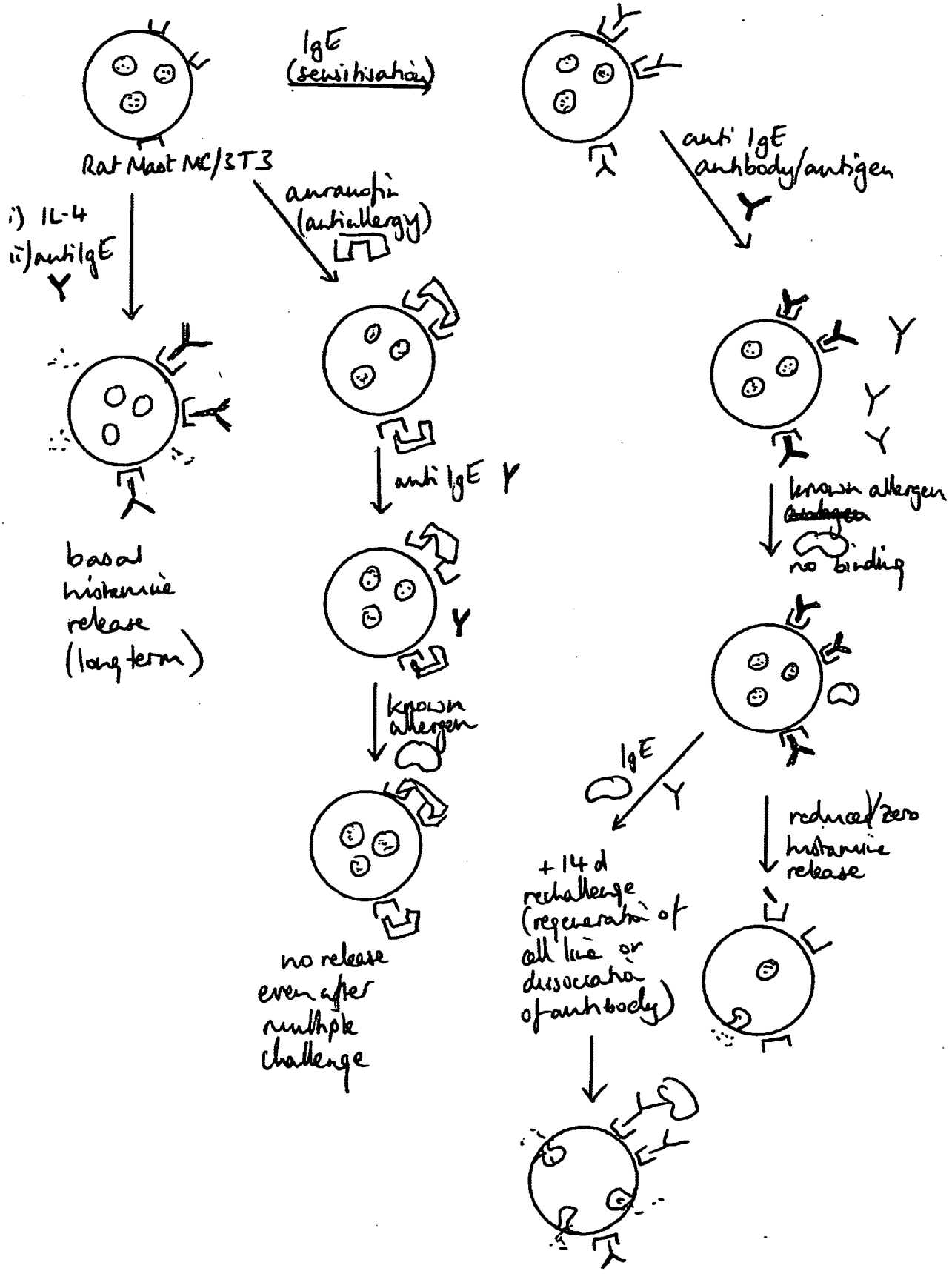

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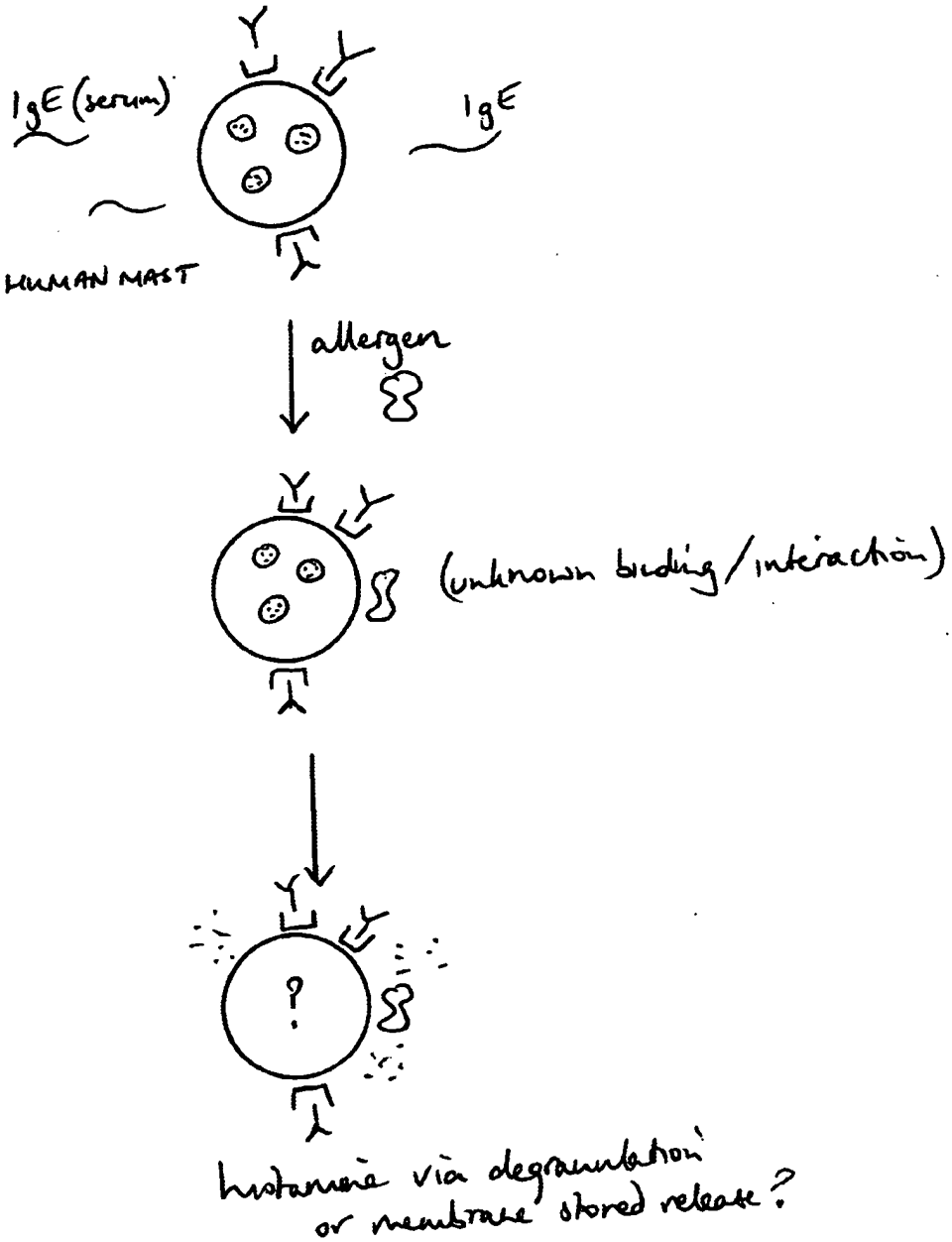
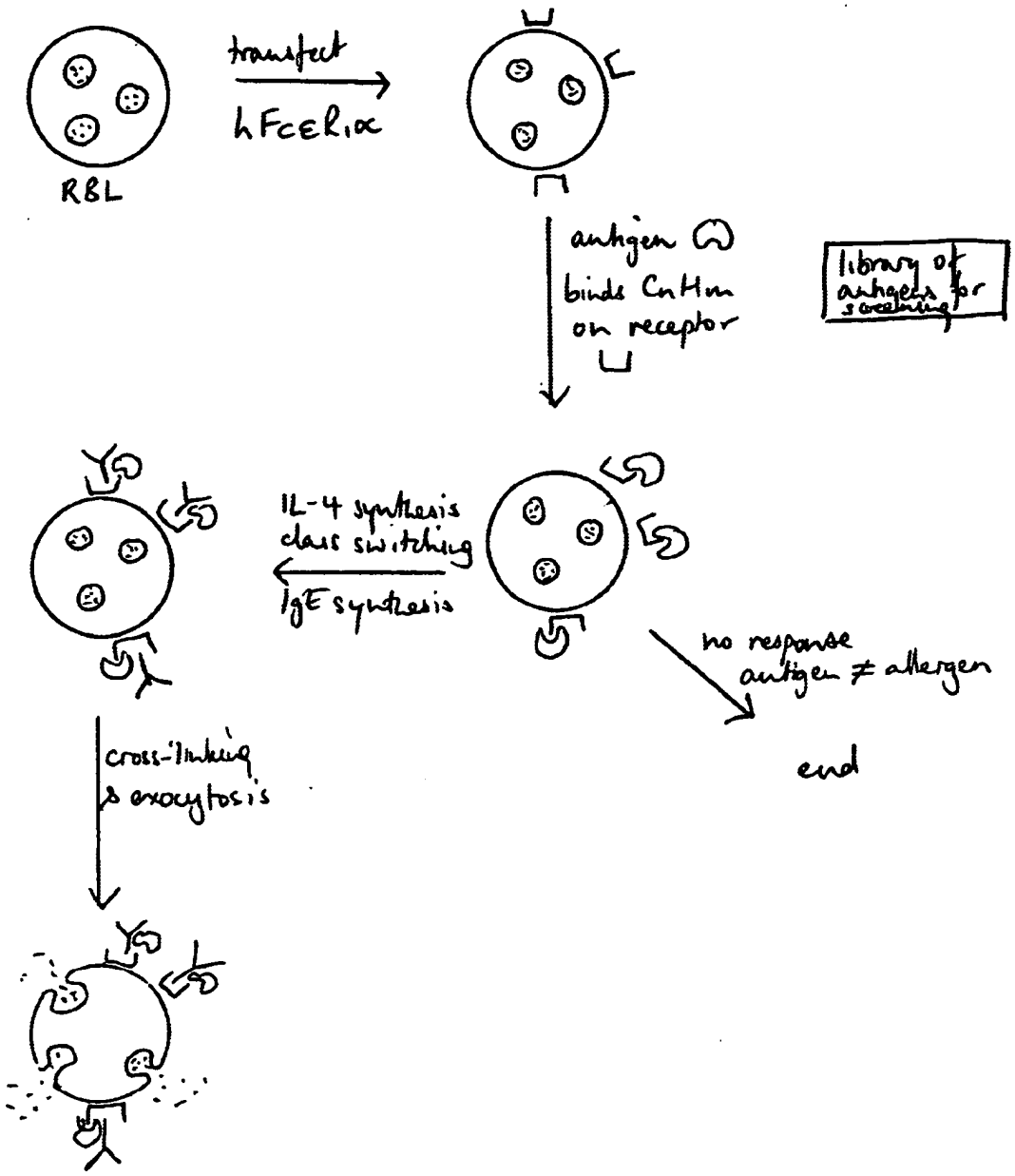


SCHEME I


RBL
transfect
hFcεRIα
antigen
binds CnHm
on receptor
library of
antigens for
screening
IL-4 synthesis
class switching
IgE synthesis
no response
antigen ≠ allergen
end
cross-linking
& exocytosis
leukotriene, histamine,
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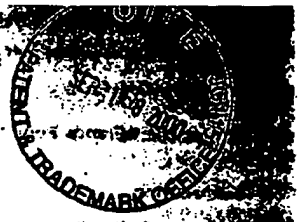
synthesis & release
antigen = allergen



MOLECULAR BIOLOGY OF THE CELL

SECOND EDITION

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

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called *secretory component* (Figure 18–19). In secretions, IgA is a dimer. It is transported from the extracellular fluid into the secreted fluid by the same kind of transepithelial transport process (transcytosis) that transfers IgG molecules from maternal blood to fetal blood. In this case the transport is mediated by a special class of Fc receptors that are present on the nonluminal surface of the epithelial cells lining the intestine, bronchi, or the milk, salivary, or tear ducts, where they bind IgA dimers present in the extracellular fluid (Figure 18–20).

The Fc region of IgE molecules binds with unusually high affinity ($K_a \sim 10^{10}$ liters/mole—see below) to yet another class of Fc receptors. These receptors are located on the surface of *mast cells* in tissues and on *basophils* (see p. 974) in the blood, and the IgE molecules bound to them in turn serve as receptors for antigen. Antigen binding triggers the cells to secrete a variety of biologically active amines (particularly *histamine* and, in some species, *serotonin*) (Figure 18–21). These amines cause dilation and increased permeability of blood vessels and are largely responsible for the clinical manifestations of such *allergic* reactions as hay fever, asthma, and hives. In normal circumstances the blood vessel changes are thought to help white blood cells, antibodies, and complement components to enter sites of inflammation. Mast cells also secrete factors that attract and activate a special class of white blood cells called *eosinophils* (see p. 974), which can kill various types of parasites, especially if the parasites are coated with IgG antibodies.

Antibodies Can Have Either κ or λ Light Chains but Not Both

In addition to the five classes of H chains, higher vertebrates have two types of L chains, κ and λ, either of which may be associated with any of the H chains. An individual antibody molecule always consists of identical L chains and identical H chains; therefore its antigen-binding sites are always identical. This symmetry is crucial for the cross-linking function of secreted antibodies. An Ig molecule, consequently, may have either κ or λ L chains, but never both. No difference in the biological function of these two types of L chain has yet been identified.

The Strength of an Antibody-Antigen Interaction Depends on Both the Affinity and the Number of Binding Sites[16]

The binding of an antigen to antibody, like the binding of a substrate to an enzyme, is reversible. It is mediated by the sum of many relatively weak noncovalent forces, including hydrophobic and hydrogen bonds, van der Waals forces, and ionic interactions. These weak forces are effective only when the antigen molecule is close

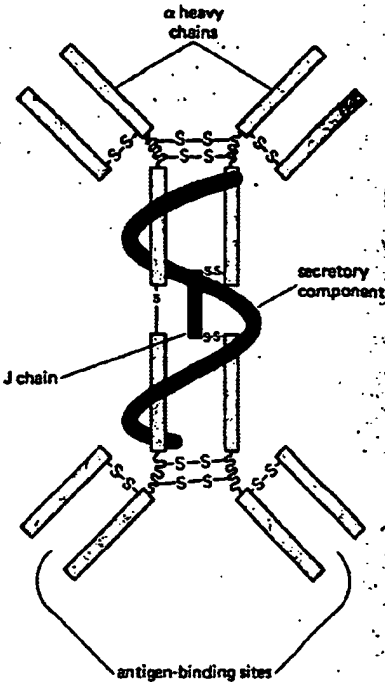

Figure 18–19 A highly schematized diagram of a dimeric IgA molecule found in secretions. In addition to the two IgA monomers that are disulfide-bonded through one of their α heavy chains, there is a single J chain and an additional polypeptide chain of 70,000 daltons called the *secretory component*, which is thought to protect the IgA molecules from being digested by proteolytic enzymes in the secretions.

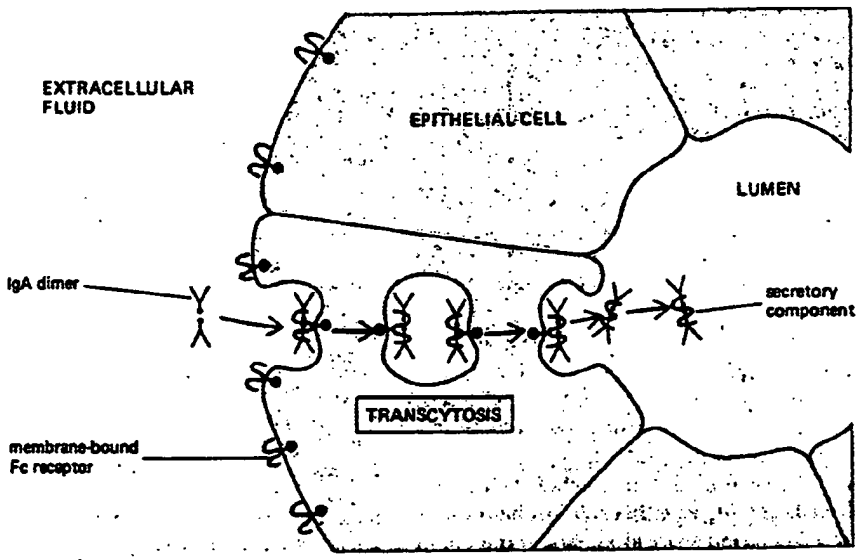

Figure 18–20 The mechanism of transport of a dimeric IgA molecule across an epithelial cell. The IgA binds to a specialized transmembrane Fc receptor protein on the nonluminal surface of the epithelial cell. The receptor-IgA complexes are ingested by receptor-mediated endocytosis, transferred across the epithelial cell cytoplasm in vesicles, and secreted into the lumen on the opposite side of the cell by exocytosis. When exposed to the lumen, the part of the Fc receptor protein that is bound to the IgA dimer (the *secretory component*) is cleaved from its transmembrane tail, thereby releasing the antibody as a complex into the lumen.

The IgA dimers enter the extracellular fluid in secretory organs from two sources. They are produced locally by IgA-secreting plasma cells in these organs, and they are produced in the spleen and lymph nodes, from where they travel in the bloodstream, leaking out of capillaries in various tissues.

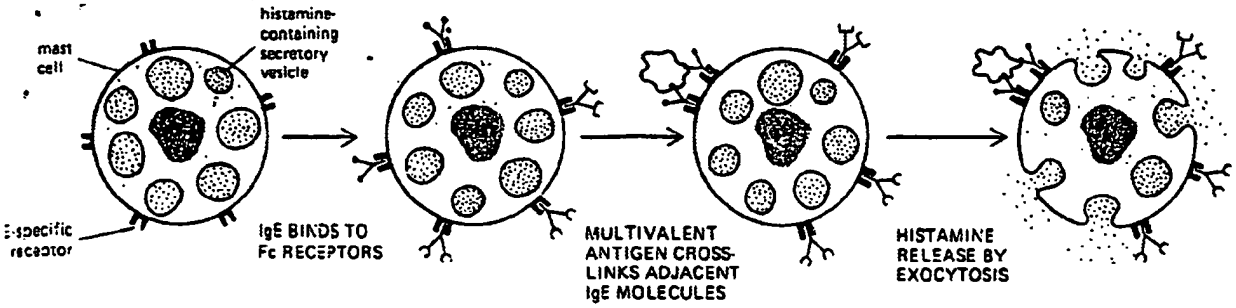

Figure 18–21 Mast cells (and basophils) passively acquire cell-surface receptors that bind antigen. IgE antibodies secreted by active B lymphocytes enter the tissues and bind to Fc receptor proteins on the mast cell surface that specifically recognize the Fc region of these antibodies. Thus, unlike B cells, individual mast cells (and basophils) have cell-surface antibodies with a variety of antigen-binding sites. When an antigen molecule binds to these membrane-bound IgE antibodies so as to cross-link them to their neighbors, it activates the mast cell to release its histamine by exocytosis.

ough to allow some of its atoms to fit into complementary recesses on the rface of the antibody. The complementary regions of a four-chain antibody unit e its two identical antigen-binding sites; the corresponding region on the antigen an *antigenic determinant* (Figure 18–22). Most antigenic macromolecules have any different antigenic determinants; if two or more of them are identical s in a polymer with a repeating structure), the antigen is said to be *multivalent* igure 18–23).

The reversible binding reaction between an antigen with a single antigenic terminant (denoted Ag) and a single antigen-binding site (denoted Ab) can be pressed as

$$Ag + Ab \rightleftharpoons AgAb$$

ie equilibrium point depends both on the concentrations of Ab and Ag and on e strength of their interaction. Clearly, a larger fraction of Ab will become sociated with Ag as the concentration of Ag is increased. The strength of the teraction is generally expressed as the **affinity constant** (K_a) (see Figure 3–7, 94), where

$$K_a = [AgAb]/[Ag][Ab]$$

ie square brackets indicate the concentration of each component at equilibrium).

The affinity constant, sometimes called the *association constant*, can be de-rmined by measuring the concentration of free Ag required to fill half of the ntigen-binding sites on the antibody. When half the sites are filled, [AgAb] = [Ab] nd $K_a = 1/[Ag]$. Thus the reciprocal of the antigen concentration that produces alf-maximal binding is equal to the affinity constant of the antibody for the an-gen. Common values range from as low as 5×10^4 to as high as 10^{11} liters/mole. ie affinity constant at which an immunoglobulin molecule ceases to be consid-ed an antibody for a particular antigen is somewhat arbitrary, but it is unlikely at an antibody with a K_a below 10^4 would be biologically effective; moreover, cells with receptors that have such a low affinity for an antigen are unlikely to activated by the antigen.

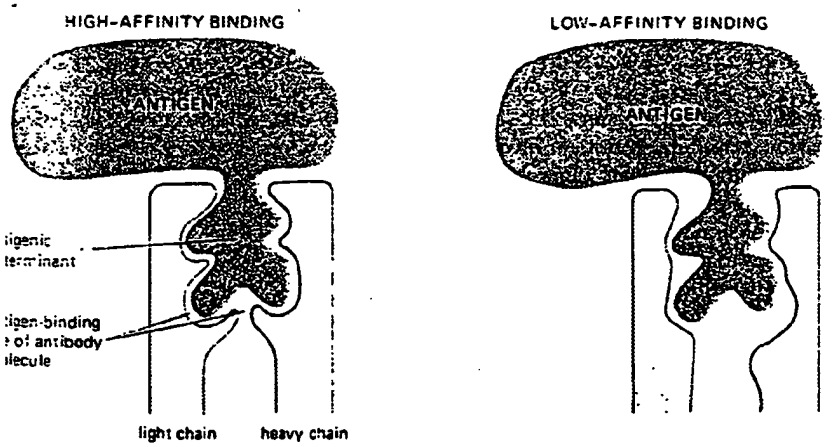

Figure 18–22 Highly schematized diagram of the binding of an antigenic determinant on a macromolecule to the antigen-binding site of two different antibody molecules, one of high and one of low affinity. The antigenic determinant is held in the binding site by various weak noncovalent forces. Note that both the light and heavy chains of the antibody molecule usually contribute to the antigen-binding site.

All classes of antibody can be made in a membrane-bound form as well as in a soluble, secreted form. The membrane-bound form serves as an antigen receptor on the B cell surface, while the soluble form is made only after the cell is stimulated by antigen to become an antibody-secreting cell. The sole difference between the two forms resides in the carboxyl terminus of the H chain: the H chains of membrane-bound IgM molecules, for example, have a hydrophobic carboxyl terminus, which anchors them in the lipid bilayer of the B cell plasma membrane, whereas those of secreted IgM molecules have instead a hydrophilic carboxyl terminus, which allows them to escape from the cell. Since a B cell contains only one copy of the C_μ gene segment per haploid genome and uses only one of its two H-chain gene pools to make antibodies, its ability to make μ chains with two types of constant regions at first seemed paradoxical. The paradox was resolved with the discovery that the activation of B cells by antigen induces a change in the way μ-chain RNA transcripts are processed in the nucleus, as explained in Figure 18–35 (see also p. 591). The switch from a membrane-bound to a secreted form of the other classes of antibodies involves a similar mechanism.

B Cells Can Switch the Class of Antibody They Make[25]

During B cell development, many B cells switch from making one class of antibody to making another—a process called **class switching.** All B cells begin their antibody-synthesizing lives by making IgM molecules and inserting them into the plasma membrane as receptors for antigen. Before they have interacted with antigen, most B cells then switch and make both IgM and IgD molecules as membrane-bound antigen receptors. Upon stimulation by antigen, some of these cells are activated to secrete IgM antibodies, which dominate the primary antibody response (see p. 1015). Other antigen-stimulated cells switch to making IgG, IgE, or IgA antibodies; memory cells express these molecules on their surface (often simultaneously with IgM molecules), while active B cells secrete them. The IgG, IgE, and IgA molecules are collectively referred to as *secondary* classes of antibodies because they are thought to be produced only after antigen stimulation and because they dominate secondary antibody responses.

Since the class of an antibody is determined by the constant region of its H chain (see p. 1021), the fact that B cells can switch the class of antibody they make without changing the antigen-binding site implies that the same assembled V_H-region coding sequence can sequentially associate with different C_H gene segments. This has important functional implications. It means that in an individual animal a particular antigen-binding site that has been selected by environmental antigens can be distributed among the various classes of immunoglobulin and thereby acquire the different biological properties characteristic of each class.

Class switching occurs by two distinct molecular mechanisms. When virgin B cells change from making membrane-bound IgM alone to the simultaneous production of membrane-bound IgM and IgD, the switch is thought to be due to a change in RNA processing. The cells produce large primary RNA transcripts that contain the assembled V_H-region coding sequence along with both the C_μ and C_δ sequences; IgM and IgD molecules are then produced by differential splicing of these transcripts (Figure 18–36). It is thought that the same mechanism underlies the switch to other classes of membrane-bound Ig when virgin B cells are stimulated by antigen to mature into memory cells that carry IgG, IgE, or IgA as antigen receptors on their surface.

By contrast, terminal maturation to an active B cell secreting one of the secondary classes of antibody is accompanied by an irreversible change at the DNA level—a process called *switch recombination.* It entails deletion of all the C_H gene segments upstream (that is, on the 5′ side as measured on the coding strand) of the particular C_H segment the cell is destined to express (Figure 18–37). Evidence that this step in class switching involves DNA deletion comes from experiments on myeloma cells: myeloma cells that secrete IgG lack the DNA coding for C_μ and C_δ regions, and those that secrete IgA lack the DNA coding for all of the other classes of H-chain C regions.

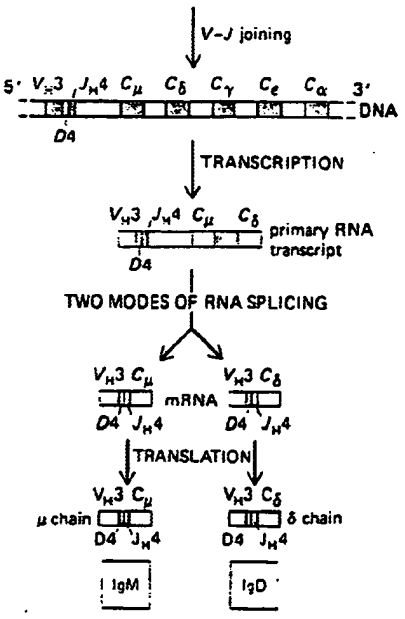

Figure 18–36 B cells that simultaneously make plasma-membrane-bound IgM and IgD molecules having the same antigen-binding sites produce long RNA transcripts that contain both C_μ and C_δ sequences. These transcripts are spliced in two ways to produce mRNA molecules that have the same V_H-region coding sequence joined to either a C_μ or a C_δ sequence. It is possible that the RNA transcripts produced by such cells are even longer than shown and contain all of the various C_H sequences.